



# Significance of Aldose Reductase in Diabetic cataract

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## ABSTRACT

**Background:** Cataract is a major cause of blindness. Diabetes mellitus is a major risk factor for the development of cataract. Diabetic patients have 25 times higher risk of cataract than non-diabetic population. The metabolic changes accompanying hyperglycemia is increased activity of the polyol pathway and aldose reductase (AR) is a key enzyme of polyol pathway. Aldose reductase is responsible for generation of more oxidative stress by decreasing GSH in diabetic patients which is thought to be a major factor to initiate the process of cataractogenesis. The present study was designed to determine significance of aldose reductase diabetic cataract and its correlation with reduced glutathione (GSH) and Malondialdehyde (MDA) in diabetic cataract patients.

**Methods:** In this study we measured MDA as oxidative stress marker & Glycated Hb (HbA1c) glycemic index marker levels of GSH and AR in erythrocytes of Type2 diabetic cataract patients (n = 30) and non diabetic senile cataract patients (n=30) compared with age matched normal controls(n=30).

**Results:** We found increased levels of AR, HbA1c and MDA, and decreased levels of GSH in diabetic cataract patients compared to non diabetic senile cataract patients and normal controls.

**Conclusions:** From the result it is concluded that AR play a major role in generation of more oxidative stress in diabetic patients which may be the cause of early cataractogenesis in diabetic patients as compared to non diabetic senile cataract patients.

**Key Words:** Cataract, Aldose Reductase (AR), Malondialdehyde (MDA), Reduced Glutathione (GSH), Glycated Hb (HbA1c).

## INTRODUCTION

Population growth, ageing, urbanization, sedentary lifestyles and an increasing prevalence of obesity are increasing the number of people with diabetes mellitus. The global prevalence of diabetes was estimated to be 2.8% in 2000 and is expected to reach 4.4% by 2030. Cataract is the leading cause of blindness in the world, responsible for 48% of blindness worldwide. A putative cause for age-related cataract is oxidative stress<sup>1</sup>. Chronic hyperglycemia is a major determinant in the development of secondary complications of diabetes, such as diabetic cataract. Evidence indicate that both the duration of diabetes and the quality of glycemic control are the most important risk factors for cataract formation<sup>2</sup>. According to WHO survey, India will be the world's diabetic capital in near future. Globally, cataract remains the leading cause of blindness, affecting approximately 18 million people. Cataract occurs at an earlier age and is 2–5 times more

frequent in patients with diabetes, thus the visual loss has a significant impact on the working population<sup>3</sup>

Diabetes mellitus is recognized as a leading located in the eye (cornea, retina, lens) is a key enzyme of polyol pathway. Under normal glycemic conditions, only a small fraction of glucose is metabolized through the polyol pathway, as the majority is phosphorylated by hexokinase, and the resulting product, glucose-6-phosphate, is utilized a substrate for glycolysis or pentose phosphate metabolism<sup>4</sup>. However, in response to the chronic hyperglycemia found in diabetics, glucose flux through the polyol pathway is significantly increased. Up to 33% of total glucose utilization in some tissues e.g. eye can be through the polyol pathway<sup>5</sup>.

In hyperglycemia excessive amount of glucose is diverted to the polyol pathway, where AR reduces glucose into sorbitol at the expense of NADPH. Sorbitol is as an osmolyte leads to osmotic swelling ,changes in the membrane permeability

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,leakage of glutathione, myoinositol ,the generation of free radicals and hydrogen peroxide which primarily causing the diabetic complications such as cataract, retinopathy & neuropathy<sup>6</sup>. Since NADPH is essential for generation of GSH (intracellular antioxidant) from GSSG, the depletion of NADPH by the AR pathway may impair intracellular antioxidant defence. Sorbitol is then converted to fructose by SDH with the production of NADH, potentially leading to increased ROS via NADH oxidase Activity of aldose reductase is dependent on NADPH. GSH is required for regeneration of NADPH. So indirectly the activity of AR is in turn depends on GSH. So in present study was designed to determine the levels of aldose reductase and GSH and their role in contribution of oxidative stress to diabetic cataract.

## MATERIALS AND METHODS:

The study comprises of total 90 subjects were divided into three groups aged between 50 -80 years. The subjects were selected from ophthalmic OPD of B. J. Medical College & Sassoon Hospitals Pune

GROUP I - (n = 30) Senile cataract patients.

GROUP II- (n =30) Diabetic cataract patients and

GROUP III- (n =30) Normal healthy controls

**Inclusion criteria** – Senile cataract subjects had normal fasting blood glucose level with no history of diabetes. Diabetic cataract subjects having diabetes for last 12-15 years and were using oral hypoglycaemic agents

**Exclusion criteria:** - Patients with ocular surgery, trauma, infection inflammation of eye, known cases of cardiovascular disorders, rheumatoid arthritis and carcinomas where free radical damages has been commonly incriminated were excluded from the study.

The study was approved by the Institutional Ethics Committee of B.J. Medical college Pune. A written informed consent was taken from the subjects.10 ml of venous blood was collected during preoperative period in plain vacutainer, EDTA and ACD (Acid Citrate Dextrose) bulb under aseptic precaution. Serum and hemolysate was used for investigations.

Blood samples of all three groups were analyzed for following parameters .

Aldose reductase activity (AR) –by Hayman and Kinoshita 1965 <sup>7</sup>

Glycated Hb (HbA1c) - by Ion exchange resin method <sup>8</sup>

Reduced glutathione (GSH) - by Beutler et al.1963 <sup>9</sup>

Malondialdehyde (MDA) - by Buege and Aust. 1978 method <sup>10</sup>

**Statistical analysis:** The data were expressed as mean  $\pm$  standard deviation. Mean values were compared by one-way ANNOVA . Differences between comparison groups were considered to be significant where  $p < 0.05$ . Post hoc donnet test for inter group comparison & correlation was used.

## RESULTS

**Table no 1:** The mean age of diabetic cataract patients ( Group II) was significantly lowered as compared to controls (Group III),and as compared to senile cataract patients (Group I) (Difference in age in between Senile and control were not statistically significant.

**Table 1: Comparison of age in study groups**

Sr. No.	Group	Age (Yrs) Mean $\pm$ SD (n=30)
1	Group I Senile Cataract	67.77 $\pm$ 3.91***
2	Group II Diabetic Cataract	58.76 $\pm$ 4.41***
3	Group III Control	66.81 $\pm$ 4.86

\*\*\*  $P < 0.0001$

Group I Vs Group II:  $P < 0.0001$ , Group II Vs Group III:  $P < 0.0001$

**Table no 2:** It is observed that the levels of HbA1C were significantly increased in diabetic cataract patients(Group II) as compared to controls (Group III) and the levels of HbA1C in diabetic cataract were significantly higher as compared to senile cataract patients

Difference of mean of HbA1C between senile and control was not statistically significant.

It is observed that activity of AR in diabetic cataract patients were significantly increased as compared to controls .Also activity of AR was significantly higher in diabetic cataract patients as compared to senile cataract patients .

There was no statistically significant difference was seen in activity of AR of senile cataract patients as compared to controls

MDA represents lipid peroxidation and act as oxidative stress marker . It is observed that levels of MDA in diabetic cataract patients (Group II) were significantly increased as compared to controls (Group III)

MDA levels in senile cataract patients (Group I) were significantly increased as compared to controls (Group III)

In diabetic cataract patients (Group II) the levels of MDA were significantly higher as compared to senile cataract patients (Group I)

It is observed that levels of reduced glutathione were significantly decreased in senile cataract (Group I) and diabetic cataract patients (Group II) as compared to controls statistically significant difference in reduced glutathione levels between senile and diabetic cataract were seen .

**Table 2: Comparison of Parameters in study groups**

GROUPS (n=30)	PARAMETERS ( Mean $\pm$ SD)			
	HbA1c %	AR units/ gHb	MDA nmol/ ml	GSH ( $\mu$ mol/g Hb)
Group I Senile Cataract	4.52 $\pm$ 0.90	3.46 $\pm$ 1.41	3.27 $\pm$ 0.29**	5.79 $\pm$ 0.92*
Group II Diabetic Cataract	9.22 $\pm$ 1.25**	4.57 $\pm$ 1.26**	4.46 $\pm$ 0.27**	4.81 $\pm$ 0.69**
Group III Control	4.68 $\pm$ 0.54	3.20 $\pm$ 1.35	2.96 $\pm$ 0.17	6.53 $\pm$ 1.21

\*\* p<0.001, \* p <0.01

**Table no 3** Showed Pearson's coefficient Correlation (r) between AR and other parameters in study group I (senile cataract patients).It is observed that There was no correlation between ,AR and HbA1c AR and MDA and AR and GSH in Senile cataract patients.

**Table 3: Correlation between AR and in study group I**

Sr. No.	Correlation between AR and	r-value	p-value
1	Age	0.13	>0.05
2	HbA1c	0.27	>0.05
3	MDA	0.025	>0.05
4	GSH	-0.004	>0.05

**Table no 4:** Showed Pearson's coefficient Correlation (r) between AR and other parameters in study group II (Diabetic cataract patients) It is observed that There is no correlation between AR and mean age and between AR and duration of diabetes of diabetic cataract patients . ii) There is highly significant positive correlation between AR and HbA1c AR and MDA in diabetic cataract patients iii) There was a significant negative correlation between AR and GSH.

**Table 4: Correlation between AR and in study group II**

Sr. No.	Correlation between AR and	r Value	P Value
1	Age	0.21	>0.05
2	Duration of diabetes	-0.12	>0.05
3	HbA1c	0.49	< 0.0001
4	MDA	0.44	<0.0001
5	GSH	-0.43	<0.0001

## DISCUSSION

In this study, we attempted to evaluate the role of Aldose Reductase in cataractogenesis of diabetic patients. In present study it is found increased levels of HbA1C, AR, MDA and decreased levels of GSH in diabetic cataract patients as compared to senile cataract patients and as compared to controls. The mechanism of cataractogenesis in diabetes is associated with oxidative stress and osmotic stress. Eye is an organ that is continuously being exposed to oxidative stress throughout the life. Aging is a major risk factor for cataract and the prevalence is slightly higher in women considering all types of cataract. Increasing age is directly related with all types of cataracts<sup>11</sup>.

The mean age of diabetic cataract patients were significantly lower as compared to senile cataract patients (Table no 1).From the results it is observed that the process of cataractogenesis occur at early an age in diabetic patients as compared to non diabetic senile cataract patients. Our results are supported by the findings of studies of Deepa K et al <sup>12</sup> and Anjuman Gul et al <sup>13</sup>.

Complications in diabetes depending on duration of diabetes and glycemic control HbA1c ( glycated Hb) is a good indicator of glycemic control . Measurement HbA1c reflects glycemic control for last 4-6 weeks

In present study the levels of HbA1c were significantly increased in diabetic cataract patients as compared to senile cataract patients and as compared to controls (Table no 2). Increased levels of HbA1c in diabetic cataract patients indicated the poor glycemic control which might be responsible for early cataract formation in diabetic patients as compared to non diabetic senile cataract patients .From this It is clear that an increased glycated haemoglobin level is associated with increased risk of cataract in patients with diabetes<sup>14,15</sup>. Our results are strongly supported by studies of various investigators M Lind et al <sup>16</sup>, Anjuman Gul et al<sup>13</sup>, Bhavna et al <sup>17</sup>.

Poor glycemic control leads to hyperglycemia and hyperglycemia induces polyol pathway. Aldose reductase is a key enzyme of this pathway.

We found high levels of AR in diabetic cataract patients as compared to senile cataract patients and as compared to controls (Table no 2). Hyperglycemia causes more & more glucose to enter in the polyol pathway which stimulates Aldose reductase (AR) that might facilitate the process of cataractogenesis.

A significant positive correlation between AR and HbA1c in diabetic cataract group (Table no 4) indicates that a significant increase in Aldose reductase activity in diabetic cataract patients due to hyperglycemia. Aldose Reductase (AR ) catalyzes the reduction of glucose to sorbitol through polyol

pathway. The sorbitol pathway is stimulated in diabetes in those tissues that do not require insulin for cellular glucose uptake, such as the retina, kidney, peripheral nerves and blood vessels<sup>18</sup>. So there is free entry to glucose in lens and retina. AR reduces glucose to sorbitol. Which accumulates in lens and exerts osmotic stress leads to cataract formation. Our results strongly support the hypothesis that hyperglycemia induces/stimulates polyol pathway at the same time the diabetes reduces age of cataractogenesis.

In our study we found significant decreased levels of GSH in diabetic cataract group as compared to controls and as compared to senile cataract patients (Table no 2).

In addition to this a significant negative correlation was found in between AR and GSH (Table 4). Hyperglycemia is characterised by increase oxidative stress. Thus hyperglycemia is also responsible for increased flux of glucose in polyol pathway increasing the AR activity. In this Sorbitol is formed by AR at the expense of NADPH. Increased activity of AR decreases the concentration of GSH as there is less availability of NADPH for generation of GSH. This is explained by figure 1. Thus these results shows that depletion in GSH level might be responsible for increased oxidative stress. Our results are supported by the work of various investigators who reported the increase in oxidative stress in diabetic cataract by Oshi et al<sup>19</sup> & G. Bhanuprakash Reddy et al<sup>20</sup>.

Oxidative stress is characterized by an increase in the concentration of free radicals which can cause damage at different levels of cellular organization. Oxidative stress causes ocular membrane lipid peroxidation as well as lens protein oxidation MDA is a indicator of lipid peroxidation<sup>21</sup>.

In our study we have observed high levels of MDA in diabetic and senile cataract patients as compared to controls. An increase in MDA levels in diabetic cataract patients is more as compared to senile cataract patients may be due to poor glycemic control because a strong significant positive correlation was found in between MDA and HbA1c in diabetic cataract patients ( $r = 0.88, p < 0.0001$ ). These findings are supported by the work of Garg et al<sup>22</sup> and Donma et al<sup>23</sup>. They showed that increased levels of lipid peroxidation product (MDA) in diabetes was due to increased production of reactive oxygen species caused by hyperglycemic status, hyperinsulemia and hyperlipidemia, which are commonly associated with diabetes<sup>24-26</sup>. This indicates that in diabetes due to hyperglycemia there is increase in lipid peroxidation which might be responsible for additional oxidative stress in diabetics. Based on the results of the AR in current study, it is reasonable to hypothesize that AR activity above the threshold level in diabetics, might be predisposed to develop cataract. Secondly polyol pathway is responsible for generation of osmotic stress and oxidative stress in diabetics

## CONCLUSIONS

From the result it is concluded that the extent of oxidative stress in diabetic cataract patients are more as compared to non diabetic senile cataract patients and AR is responsible for that generation of more oxidative stress. More oxidative stress may be the cause of early cataractogenesis in diabetic patients as compared to non diabetic senile cataract patients.

Further studies are required for therapeutic use of ARI (Aldose reductase inhibitors) to diabetic patients which may be beneficial in delaying the cataract formation in diabetics.

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## Declarations

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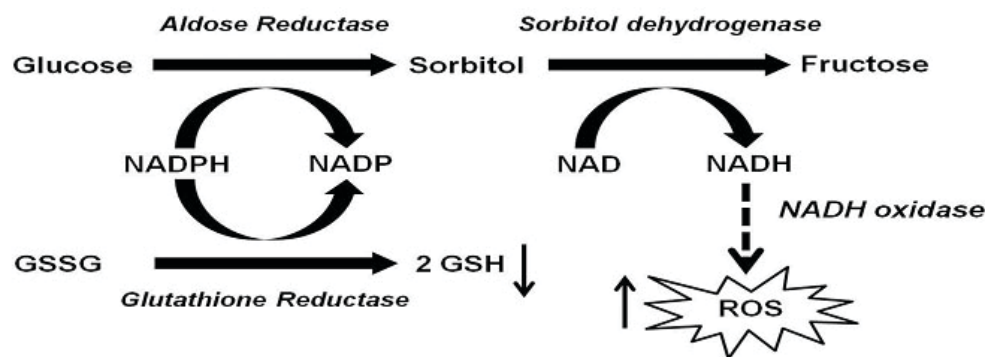
**Ethical approval:** The study was approved by the institutional ethics committee of B.J. Medical college Pune.

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**Figure 1:** Role of aldose reductase (AR) in hyperglycemia-induced oxidative stress. Excessive amount of glucose is shunted to the polyol pathway, where AR reduces glucose into sorbitol at the expense of NADPH. Since NADPH is essential for generation of GSH (intracellular antioxidant) from GSSG, the depletion of NADPH by the AR pathway may impair intracellular antioxidant defense. Sorbitol is then converted to fructose by SDH with the production of NADH, potentially leading to increased ROS via NADH oxidase.